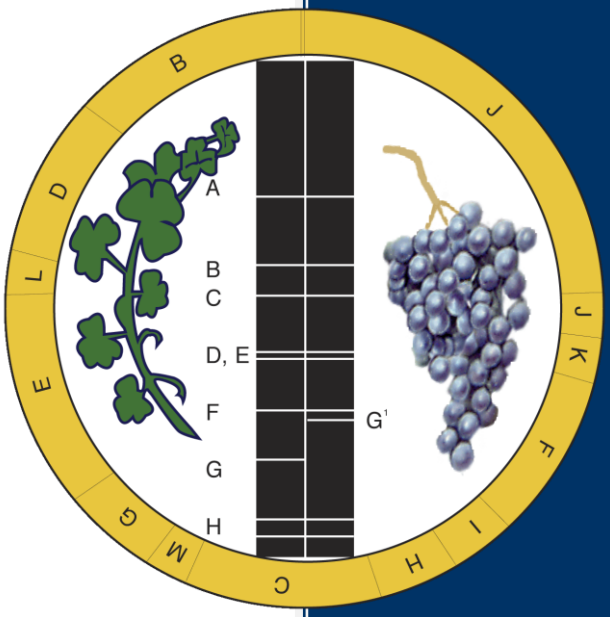
ANNUAL REPORT 2024



National Meticillin-Resistant *Staphylococcus aureus* Reference Laboratory

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INTRODUCTION

The National MRSA Reference Laboratory (NMRSARL), based at St. James's Hospital, Dublin, continues to play a pivotal role in the surveillance, investigation, and control of methicillin-resistant *Staphylococcus aureus* (MRSA) and related Gram-positive organisms in Ireland. In 2024, the laboratory experienced a further increase in workload and complexity of investigation requests, reflecting its expanding remit and the evolving epidemiology of antimicrobial resistance in MRSA.

Key Public Health Contributions

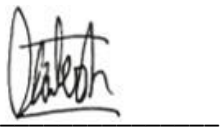
- Over 1,300 isolates were investigated, including 121 MRSA bloodstream isolates.
- Monitored outbreaks of PVL-positive S. aureus, MRSA, and vancomycin-resistant Enterococcus faecium (VRE), contributing to national and European surveillance efforts.
- Tracked resistance trends in MRSA, MSSA, CoNS, and Enterococci, with a focus on emerging resistance to linezolid, daptomycin, and other newer agents.
- Notably, linezolid resistance mechanisms (e.g., cfr, optrA, poxtA, and 23S G2576T mutations) were detected in multiple isolates.
- Whole Genome Sequencing was used extensively for outbreak investigations and epidemiological typing, including cgMLST analysis of ST22-MRSA-IV and other lineages.

Epidemiological Insights

- While still predominant, ST22-MRSA-IV decreased to 34% of MRSA bloodstream infections. This strain has predominated since the late 1990s but in recent years has significantly decline.
- Strains previoulsy considered of community origin are more frequently recovered in healthcare settings and have caused several outbreaks. These strains frequently carry increased resistance and virulence genes.
- PVL detection remained the most requested test. Among MRSA investigated, 20% of isolates were positive while 10% of MSSA were positive.
- Among MRSA and VRE investigated by WGS, multiple clusters were identified within and between heatlhcare settings.

We would like to thank the staff of NMRSARL who continue to work tirelessly to provide the service; our collaborators in research and development which yields a fruitful new knowledge on MRSA and the Laboratory Medicine Directorate and St. James's Hospital for continuing to support the NMRSARL in the important work that it provides.

We hope that you find the following pages useful and informative.



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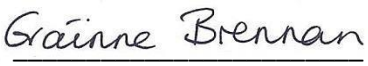

Dr. Gráinne Brennan
Chief Medical Scientist

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ROLE OF THE LABORATORY

Since its establishment in 2002, the Laboratory has supported efforts to prevent and control the spread of MRSA in Ireland, by providing expertise to laboratories in the correct identification of *Staphylococcus aureus* isolates, by tracking circulating strains as part of infection control, by detecting the emergence of new mechanisms of resistance to antibiotics, by screening for the presence of novel virulence factors or toxins, and by participation in research and development initiatives at home and abroad.

SERVICES

The NMRSARL provides the following services:

- Investigation of MRSA isolates using phenotypic and molecular techniques for the following reasons:
  - confirmation of *S. aureus* identity
  - epidemiological typing (including *spa* typing)
  - detection of resistance and virulence genes including *pvl, mec, nuc, eta, etb* and *etd*
- Investigation of meticillin susceptible *S. aureus* (MSSA) isolates
  - For the detection of the *pvl* and exfoliative toxin genes
  - Outbreak investigation of strains using *spa* typing
- Epidemiological typing of *Enterococcus faecium* and detection of linezolid resistant determinants
- Advice
  - on treatment and management of patients with MRSA through its medical director
  - on infection control through the infection control team of SJH
  - on laboratory aspects of MRSA through the scientific staff of the laboratory.

ISOLATES

Isolates, recovered from patients attending community medical practitioners or hospitals, are submitted to the laboratory from all hospital microbiology laboratories throughout the Republic of Ireland.

In addition to this, the NMRSARL also provides laboratory support for the MRSA component of EARS-Net in Ireland. All Irish hospital laboratories participating in EARS-Net send MRSA isolates from blood cultures (one per patient per year) to NMRSARL where they are investigated for resistance to oxacillin, vancomycin and teicoplanin using standard gradient MIC strip and macro-method techniques. NMRSARL also provides data on rates of resistance to other clinically useful antibiotic

PUBLIC HEALTH IMPACT

The impact of the various activities of the NMRSARL on public health is described below.

Organism	Activity	Number of isolates	Outcome
MRSA blood culture isolates	Surveillance	121	Participation in EARS-Net which is a European wide network of national surveillance systems, providing European data on antimicrobial resistance for public health purposes
MRSA & MSSA	PVL toxin testing	906	Surveillance, recognition, investigation and management of PVL *S. aureus* in Ireland
MRSA & MSSA	Surveillance analysis and identification of trends	1172	Typing and susceptibility testing of MRSA and MSSA isolates submitted throughout the year.
MRSA and MSSA	Confirmation of resistance against various antibiotic agents	737	Confirmation of resistance against glycopeptides, β-lactams, daptomycin and newer agents.
VRE and CoNS	Confirmation of linezolid and other resistance	132	Characterisation of resistance mechanism associated with increased resistance in VRE and CoNS

REFERENCE LABORATORY WORK

As expected, MRSA isolates accounted for the largest proportion (43.2%) of the laboratory workload during 2024. Isolates recovered from bloodstream infections that were investigated under the European Antimicrobial Resistance Surveillance Network (EARS-Net) accounted for 8.5% of the overall workload. As in previous years the number of requests to investigate susceptible *S. aureus* and other Gram-positive organisms have also increased now representing 29.3% and 27.6% respectively (Fig. 1).

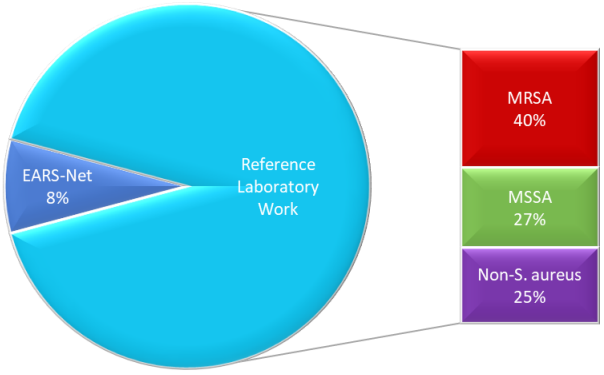


Fig 1 Workload of the NMRSARL during 2024

In recent years an increase in requests for investigations of MSSA isolates has led to a change in the services of the laboratory and 2024 saw a further increase in the uptake of newer services including the investigation of linezolid resistance among Enterococci and CoNS.

Along with a steady increase in the number of isolates submitted, the complexity of tests has also increased over time. Currently the laboratory performs phenotypic investigation on all staphylococci submitted using disk diffusion whilst all enterococci undergo MIC determination using broth microdilution. Further molecular investigation is also performed on the majority of isolates including investigation for resistance and virulence genes (n=1052) or *spa* typing (n=664). This change is primarily due to the changing epidemiology of MRSA circulating in Ireland and the limited information that can be obtained from phenotypic investigation of these emerging strains.

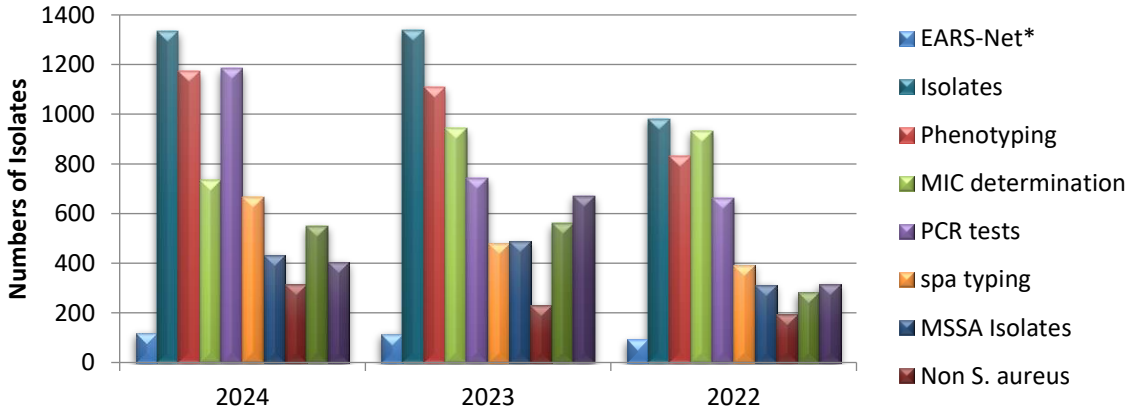


Fig 2 Distribution of workload throughout 2024

*Investigation of EARS-Net isolates received during 2024 included phenotyping, MIC determination and whole genome sequencing (WGS).

ANTIMICROBIAL RESISTANCE AMONG MRSA IN IRELAND

The phenotypic epidemiological typing techniques used in the NMRSARL enables the laboratory to monitor resistance among MRSA strains against clinically useful antimicrobial agents and to identify emerging resistance that may cause concern into the future with the EARS-Net isolates providing a representative collection of isolates recovered throughout the country. For over the last decade, the predominant strains circulating in Ireland was ST22-MRSA-IV and exhibits a non-multiantibiotic resistant susceptibility profile. However, in 2024 the prevalence of ST22-MRSA-IV further decreased to 34.2% among the EARS-Net isolates with the other lineages exhibiting greater resistance to aminoglycosides and tetracycline.

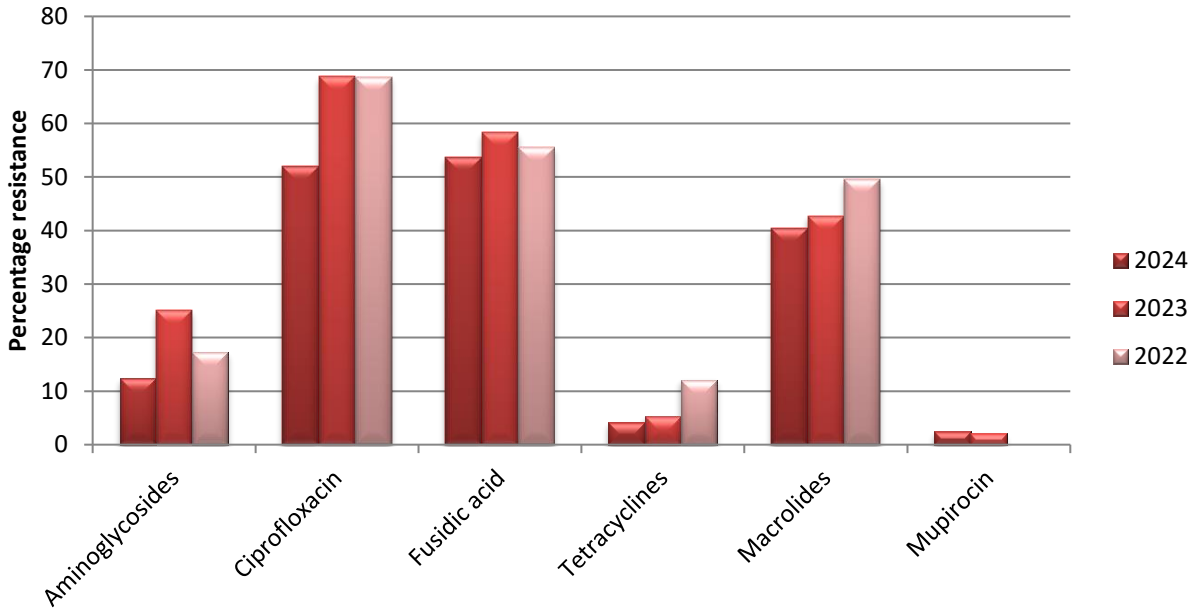


Fig 3 Resistance rates among EARS-Net isolates recovered in 2024

Antimicrobial susceptibility among MRSA recovered from non- blood stream infections

While the previously mentioned rates of resistance relate only to EARS-Net isolates, a greater proportion of the work in the NMRSARL relates to isolates recovered from non-blood stream infections. In addition, these isolates are often recovered from patients in the community where no risk factors for MRSA infection are present.

These isolates are submitted from different users on an ad hoc basis and therefore do not represent true prevalence characteristics of strains in the community. However, it is possible to determine resistance profile of the isolates that were selected for submission to the NMRSARL.

Below shows the profile of all non-BSI isolates investigated in comparison to those of BSI isolates. Whilst the ST22-MRSA-IV exhibits a non-multiantibiotic resistant profile many isolates recovered from non-BSI both in healthcare facilities and in the community, and which may also be among others, ST22-MRSA-IV, exhibit higher levels of resistance against the panel of antibiotics tested with 60% of isolates exhibiting multi-antibiotic resistance, that is, resistance to three or more different classes of antibiotics.

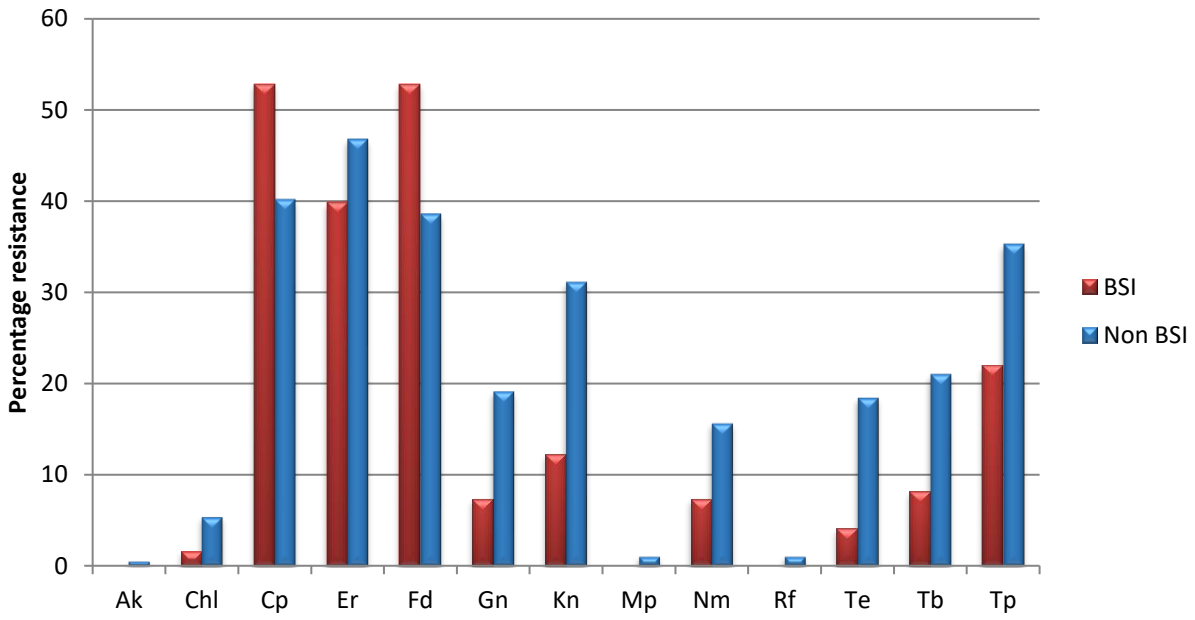


Fig 4 The percentage of blood stream MRSA isolates exhibiting resistance to each antimicrobial agent investigated in 2024 in comparison to those recovered from non-blood stream infections.

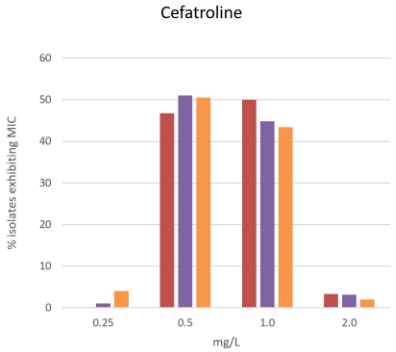
Resistance patterns determined for MRSA isolates by antibiogram- resistogram typing. Percentage for each agent includes those exhibiting resistance as determined in accordance with EUCAST or in-house developed interpretive criteria. Abbreviations: Ak; amikacin, Chl; chloramphenicol, Cp; ciprofloxacin, Er; erythromycin, Fd; fusidic acid, Gn; gentamicin, Kn; kanamycin, Mp; mupirocin, Nm; neomycin, Rf; rifampicin, Te; tetracycline, Tb; tobramycin, Tp; trimethoprim.

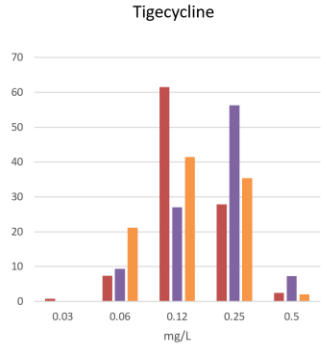
Antimicrobial resistance to newer agents

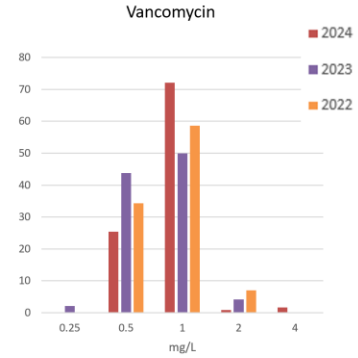
Surveillance studies provide important information in the identification of trends in the development of antimicrobial resistance. Monitoring of newer agents for treatment of MRSA infection is important as resistance detection is rare and difficult with not all laboratories routinely testing against these agents.

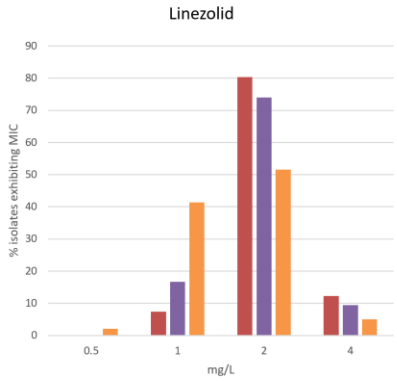
Whilst the NMRSARL has monitored susceptibility to several agents using gradient MIC strips for several years, in 2019 the laboratory introduced broth microdilution investigation for linezolid, daptomycin, ceftaroline, dalbavancin, vancomycin and tigecycline. Broth microdilution is highly accurate method for MIC determination and is often considered the gold standard of susceptibility testing.

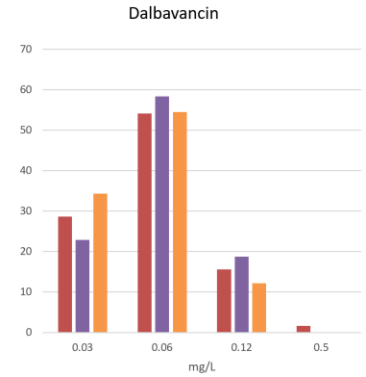
The MIC was determined by broth microdilution on all isolates submitted as part of the EARS-Net project. The distribution of the MICs observed for each agent is shown below and is compared to the MIC observed for isolates from 2020.

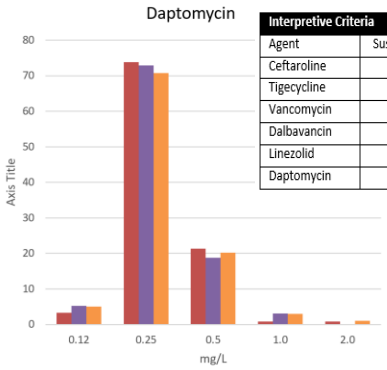












Interpretive Criteria		
Agent	Susceptible <=	Resistant >
Ceftaroline	1.0	1.0
Tigecycline	0.5	0.5
Vancomycin	2.0	2.0
Dalbavancin	0.125	0.125
Linezolid	4.0	4.0
Daptomycin	1.0	1.0

Linezolid resistance among Staphylococci and Enterococci

In 2019 Ireland had one of the highest proportions of vancomycin resistant *Enterococci faecium* (VRE*fm*) in Europe. In addition, in recent years an increase in resistance to linezolid has also been reported. Since 2016 the NMRSARL has investigated linezolid resistance in Enterococci and Staphylococci for the presence of *cfr* and *optrA* (2). In 2018 this was expanded to include the gene *poxtA* with the mutation G2576T included to the list of resistance determinants investigated in 2021 (3).

Linezolid is often the drug of last resort to treat serious infections caused by Gram-positive cocci. While resistance frequently arises due to mutations in the 23S rRNA gene, altering the drug binding site, and/or the 50S ribosomal proteins L3, L4 and L22, impairing linezolid binding, less frequently it has also been associated with the acquisition of a plasmid-encoded methyltransferase gene *cfr* or ABC transporter gene *optrA.* The presence of *cfr* can result in the $PhLOPS_A$ phenotype i.e., resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A compounds, due to their overlapping binding sites. In contrast however, *optrA* confers resistance to oxazolidinones and phenicols only while, along with these *poxtA* also encodes resistance to tetracyclines.

Work carried out in the NMRSARL has found isolates recovered in Ireland can carry multiple resistance mechanisms. In 2017 an *E. faecium* isolate was found to harbour both *cfr* and *optrA* while in 2018 an isolate was found to be positive for *optrA* and *poxtA.*

During 2024, there were 146 isolates investigated for these resistance genes. Among 132 isolates investigated, 77.3% (102/132) exhibited an MIC of ≥4.0mg/L. Among the isolates tested, *cfr* was found to be present in these 3.9% (4/132.), 13.7% (14/102) harboured *optrA* and 13.7% (14/102) were found to harbour *poxtA.* One isolate harboured both *poxtA* and *optrA.* Detection of mutational resistance associated with 23S rRNA was also investigated the G2576T mutation was identified in 62.7% (64/102) of isolates. Combinations of the G2576T mutation and either *cfr, optrA* or *poxtA* was seen in 3.9% (4/102) of isolates. Of isolates exhibiting a MIC of 4.0mg/L which, in accordance to EUCAST criteria would be considered susceptible, five isolates (35.7%, 5/14). Of these five, three harboured *optrA,* one harboured *poxtA* and the remaining one had the G2576T mutation.

EPIDEMIOLOGICAL TYPING OF MRSA IN IRELAND

For several years, the NMRSARL has used phenotypic and molecular epidemiological typing techniques. Molecular techniques include *spa* typing, which has been shown to have good concordance and congruence with MLST and enable the NMRSARL to report inferred MLST data based on the *spa* type. Since 2019 however, all isolates submitted to the NMRSARL for investigation under the EARS-Net project also undergo whole genome sequencing.

Whole genome sequencing (WGS) found that, like previous years, ST22-MRSA-IV continues to be the predominant strain circulating in healthcare settings this predominance is decreasing and an increase in the diversity of strains continues to be observed. This strain is also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13, or Canadian MRSA-8 and has been the increasing in Ireland since the late 2000s (4).

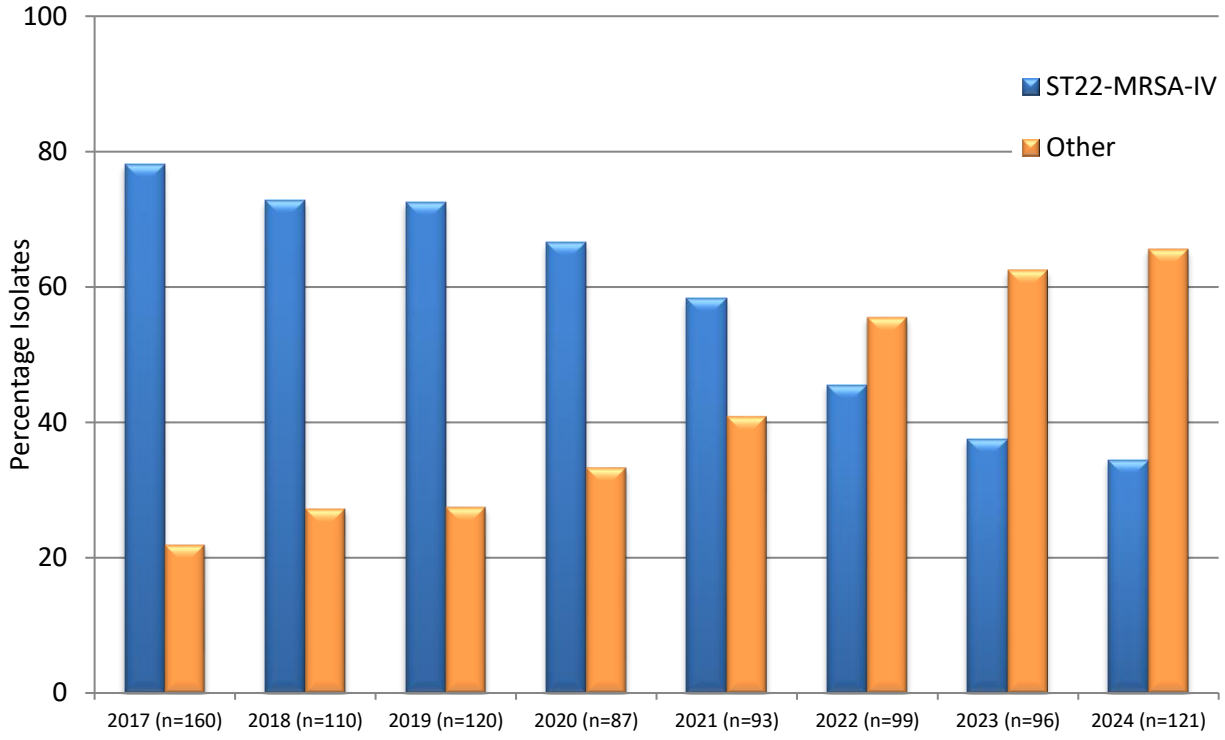


Fig 6 Epidemiological types of MRSA strains recovered from blood stream infections. 2017-2024. 2012-2018 MLST types inferred using *spa* typing and antibiogram resistogram (AR) typing.

Furthermore, in Ireland over time, a strain displacement has occurred resulting in the ST22-MRSA-IV predominating in healthcare facilities. This displacement has also been reported in other countries where, once community associated strains have now become the predominant hospital associated strains (USA 300 in America and ST772 in India). Many of the strains recognised in Ireland have been reported elsewhere and very often, these strains exhibit greater resistance and harbour more virulence genes than the ST22 strains. Hence close monitoring is required to control the spread of these strains in the hospital setting.

Unlike in previous years when all non-ST22-MRSA-IV were classed as "other", WGS has also enabled us to determine the genetic profile of these strains. These included ST1, ST5, ST8, ST30, ST97 and ST6. Information about these strains is limited due to the infrequency in which they are reported however in Ireland:

- Many of these strains are frequently associated with CA-MRSA;
- Whilst the SCC*mec*IV element continues to be the most common, SCC*mec*V was also frequently recognized among these strains
- There was increased diversity among the MRSA strains recovered from blood stream infections.

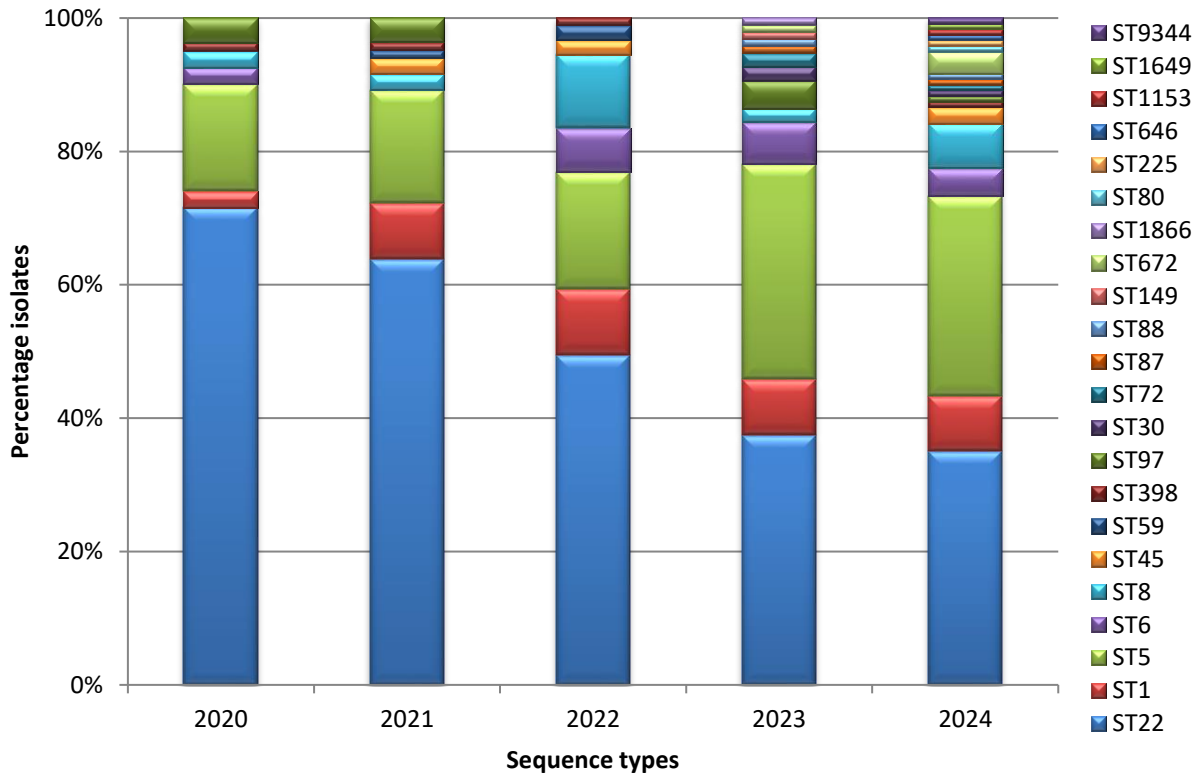


Fig 7 Epidemiological types of MRSA strains recovered from blood stream infection recovered in 2020-2024 investigated by whole genome sequencing analysis.

ST22-MRSA-IV: EPIDEMIC STRAIN PREVALENT IN IRELAND

ST22-MRSA-IV is the pandemic clone in Ireland as it is in Europe but prevalence has been decreasing in prevalence in recent years and was associated with only 34% of MRSA causing blood stream infections. This strain is also known as UK-EMRSA-15, Barnim Epidemic Strain, Spanish PFGE type E13 or Canadian MRSA-8 (4).

This strain has been reported in many countries and, where present, tends to be the predominate strain accounting for >50% of MRSA in Portugal, and Malta and in England it is currently associated with 85% of bacteraemia cases. The strain occurs in hospitals as well as among outpatients in the community and has also been recovered from companion animals such as horses, cats and dogs (4).

Due to the low discriminatory power of current bacterial epidemiological typing techniques such as *spa* typing when differentiating ST22-MRSA-IV in Irish hospitals, the NMRSARL utilized whole genome sequencing technology to provide detailed analysis of the ST22-MRSA-IV isolates (n=42) recovered from blood cultures during 2024.

Core genome multi locus sequence typing (cg-MLST) is an allele-based approach used to interpret whole genome sequencing data. cgMLST involves the comparison of 1,861 core genes and allows clustering of closely related isolates. For MRSA, whilst there are no definitive cgMLST thresholds for assigning isolate relatedness, a difference of ≤24 alleles may be used as an approximate clonality guideline. Among the 2024 EARS-Net collection, there were seven occasions where isolates had fewer than 24 differences several of which involved isolates from different hospitals.

A maximum-likelihood phylogenetic tree was constructed to illustrate the ancestral relationships between the ST22-MRSA-IV isolates based on a core genome alignment. The tree was annotated with the distribution of all identified resistance genes (Fig 8).

Common resistance patterns exhibited by the ST22-MRSA-IV strain include resistance to fusidic acid, ciprofloxacin, and erythromycin. Associated resistance genes detected included *blaZ* (β-lactamase), *erm*(C)/*lnu*(A) (macrolides) and *ant*(4)/*aph*(2) (aminoglycosides) (Fig 8). Separately, other mutational resistance determinants recognised included *fusA* (fucidic acid) and *gyrA* (ciprofloxacin). Variable virulence markers detected ST22-MRSA-IV are *sec* and *sel* as well as the IEC genes encoded by lysogenic β-haemolysin-converting phages (*sak, chp, scn*).

A separate report has been sent to each hospital detailing the results of isolates submitted from their laboratory.

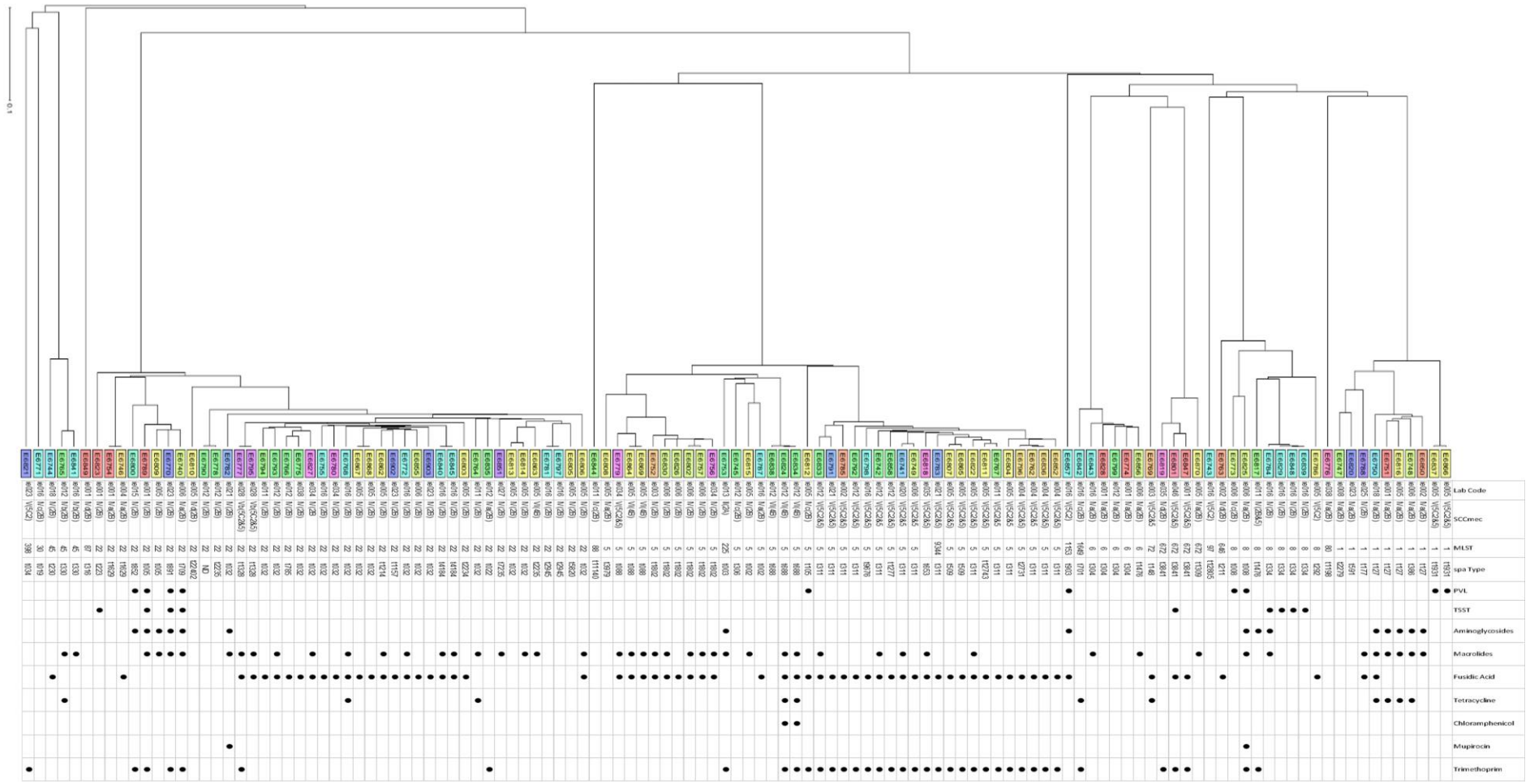


Fig 8 Phylogeny of MRSA isolates recovered from blood culture specimens during 2024. Coloured labels on tree represent different hospitals identified using their EARS-Net code which is listed in the lab code column. The phylogenetic tree was annotated with the distribution of selected resistance genes and where the gene was found to be present when there was >90% coverage of the gene at >30x depth of sequencing reads along with the *spa* type, SCC*mec* type, the MLST and the presence of PVL and TSST.

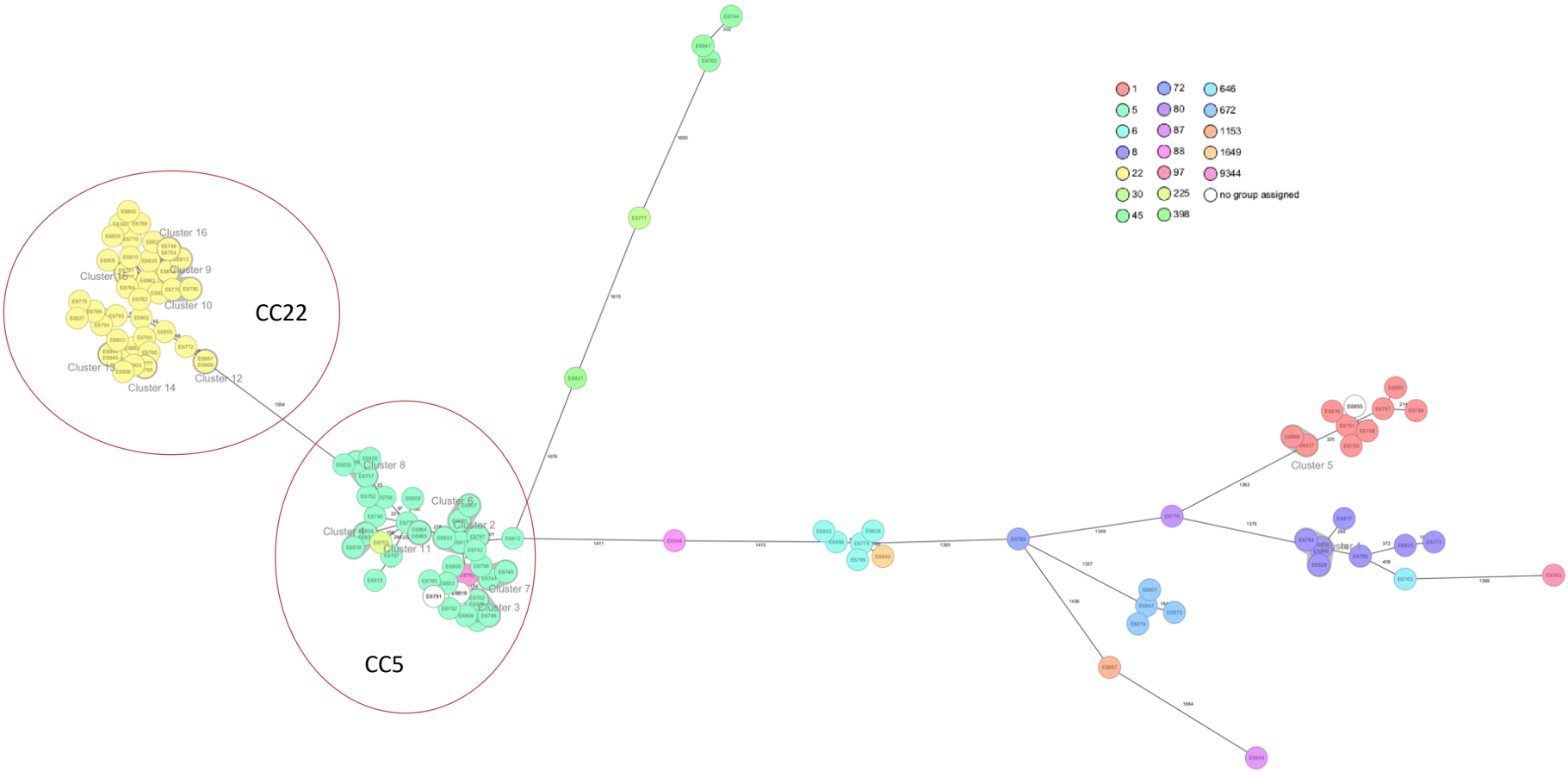


Fig 9: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates recovered from blood stream infections during 2024. MRSA isolates assigned to the same sequence type (ST) are assigned the same colour. Closely related clusters of isolates (≤24 cgMLST allelic differences) are outlined with grey shadowing. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates. Most clusters of closely related isolates were identified among those isolates assigned to CC22 and CC5.

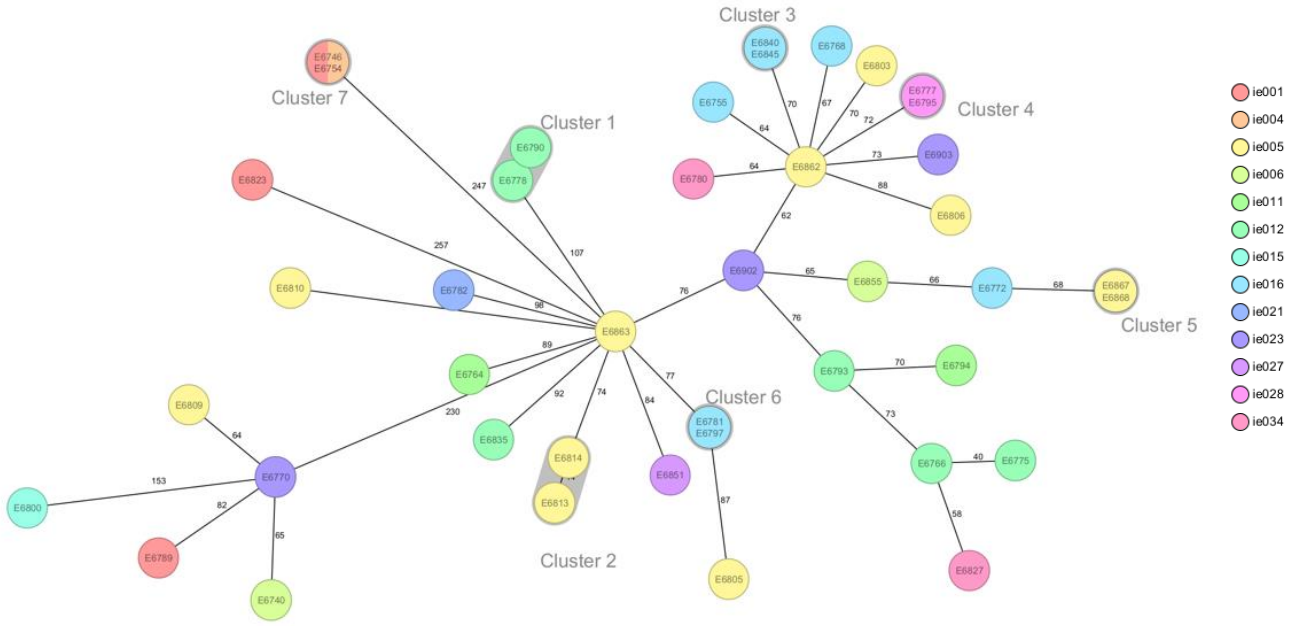


Fig 10: A minimum spanning tree constructed based on 1,861 core genomes from the ST22 MRSA isolates (n=42) recovered from blood stream infections in 2024. Each colour represents a different hospital. Isolates exhibiting ≤24 allelic differences clustered together.

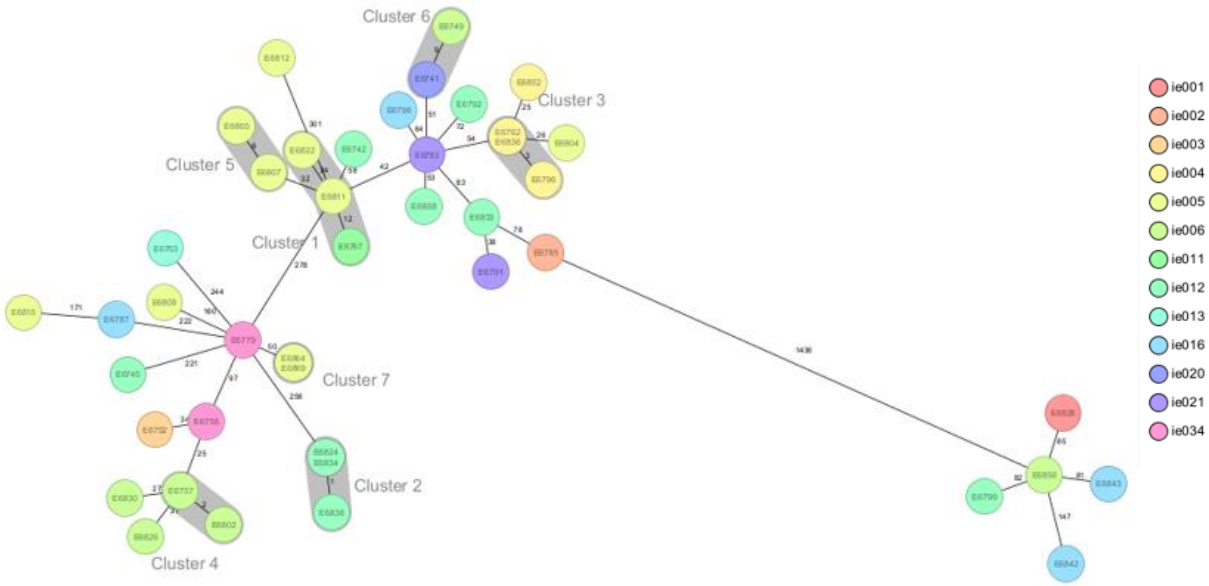


Fig 11: A minimum spanning tree constructed based on 1,861 core genomes from the CC5 MRSA isolates (n=43) recovered from blood stream infections in 2024. Each colour represents a different hospital. Isolates exhibiting ≤24 allelic differences clustered together.

PVL positive *S. aureus*

Throughout 2024 the detection of PVL continued to be the most frequently requested test. The PVL toxin is a cytotoxigenic toxin produced by *S. aureus* which is clinically associated with skin and soft tissue infections but is rarely reported in isolates recovered from invasive infections. In 2024, 906 *S. aureus* isolates (non-BSI) were investigated for carriage of the *lukS-PV* and *lukF-PV* genes encoding for PVL. The isolates investigated included 569 MRSA and 337 MSSA.

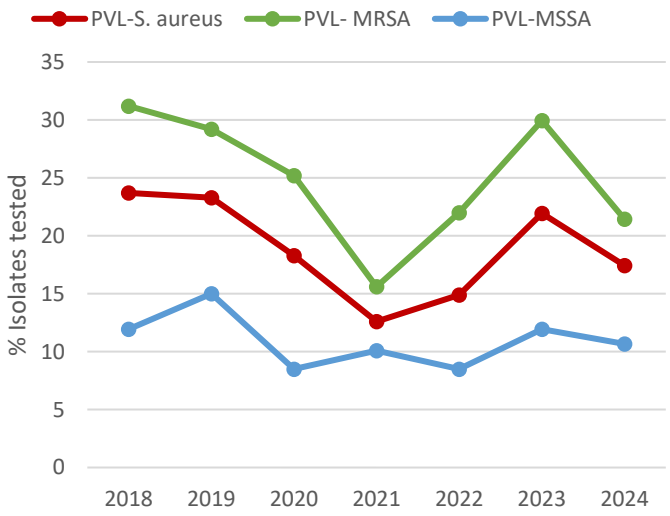


Fig 12: Frequency of PVL *S. aureus*

Among the MRSA isolates 21.4% (122/569) were found to be positive while 10.7% (36/336) of MSSA isolates were also positive.

The change in the number of PVL-positive MRSA in recent years was primarily due to several outbreaks and clusters in healthcare settings investigated during previous years.

As in previous years, the distribution of epidemiological types among PVL+ *S. aureus* is limited with less diversity seen among the MRSA isolates. In 2024, 71% % of the isolates were limited to only eight sequence types with a further the 18% not assigned to any MLST by *spa* typing (Fig 13).

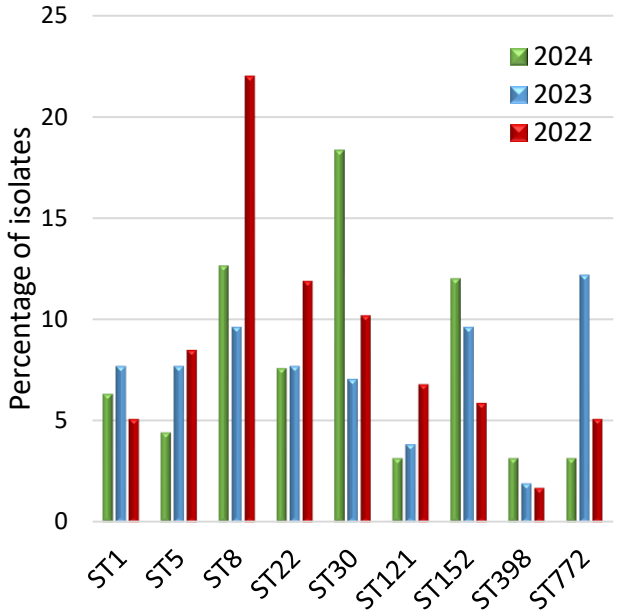


Fig 13 Distribution of sequence types among PVL-*S. aureus* isolates recovered in 2023.

ST8, ST30 and ST152 were the most frequently recognized *spa* types, all of which are strains which have been reported globally.

MOLECULAR EPIDEMIOLOGICAL TYPING OF MRSA

Typing methods for discriminating different bacterial isolates are essential epidemiological tools in infection prevention and control. Traditional methods based on phenotypic characteristics have been used for many years however often fail to provide sufficient discrimination of isolates in outbreak situations. Whilst EARS-Net undergo WGS, resource constraints limit the number of additional isolates which undergo sequencing. However, a large proportion of isolates undergo *spa* typing on an annual basis allowing easier comparison of MRSA recovered in Ireland with those recovered elsewhere throughout the world.

spa typing involves sequencing of the Staphylococcal protein A gene (*spa)* to recognise mutations or repeat insertion/deletion events that can cause changes in the polymorphic X region of the *spa* gene. It has become a well-established discriminatory method for outbreak investigations but has also been shown to be useful for long-term epidemiological studies. The availability of MLST data associated with *spa* types on an online database facilitates comparison of Irish isolates with isolates from all other countries. Based upon repeating patterns (BURP) analysis clusters *spa* types together based on the repeat succession pattern of *spa* types (5) .

Using the inferred MLST data available from the *spa* typing online database the most frequently recognised MLST types accounted for over 50% of the isolates and, like previous years, included ST1, ST5, ST8 and ST30 (Fig 14).

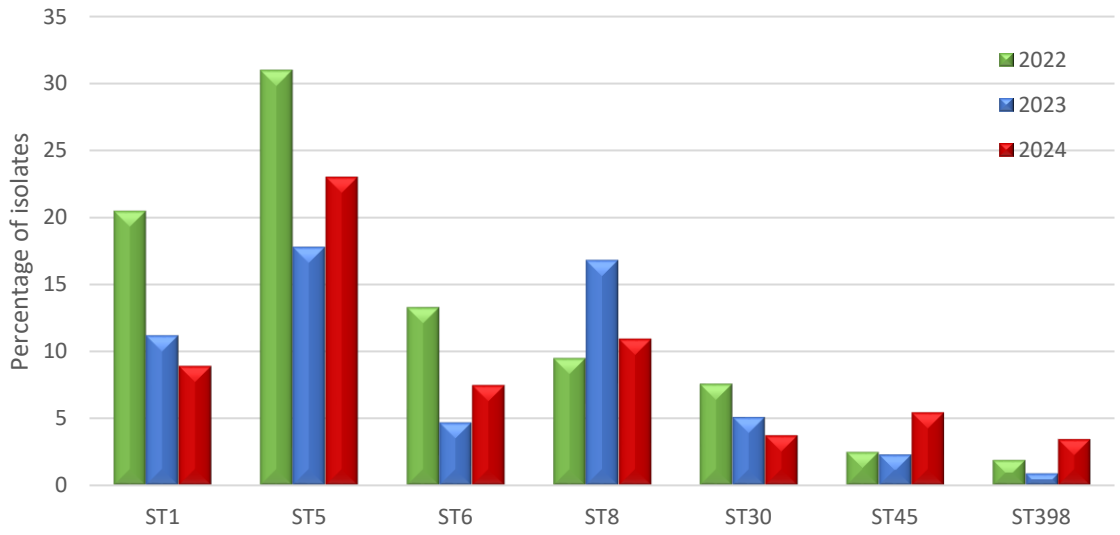


Fig 14 Most frequently recognised MLST among PVL negative MRSA isolates investigated by *spa* typing during 2024

***Sequence type inferred from data available on the Ridom *spa* typing database. Inferred MLST were not available for 39% of *spa* types (n=136) recognised.**

WHOLE GENOME SEQUENCING TO INVESTIGATE CA-MRSA LINEAGES RECOVERED IN IRISH HEALTHCARE SETTINGS

In recent years, the NMRSARL has been involved in several studies investigating the emergence of different lineages of MRSA in Ireland especially those which have been associated with outbreaks in healthcare facilities (6, 7).

Since 2019, many of these strains associated with outbreaks have undergone whole genome sequencing. Below shows an MST of all strains associated with CA-MRSA which have undergone whole genome sequencing in the NMRSASRL (Fig 12). Previously MRSA was clearly defined as healthcare associated (HCA-), community acquired (CA-) or livestock associated (LA-) however the lines of separation are becoming increasingly blurred with the importation of CA-MRSA strains into healthcare facilities and the zoonotic spread of LA-MRSA to humans. In addition, there is increasing diversity in the lineages of MRSA being recovered in Ireland.

As shown in Fig 12, multiple outbreaks were recognised among CA-MRSA lineages, which included isolates spanning several years and were in both community and healthcare settings. The largest clusters were caused by isolates from ST1, ST5, ST6 and ST772.

- t304-ST6-MRSA-IV: This strain has been recovered from a number of hospitals and increased in prevalence in recent years now accounting for 1.5-2% of non ST22 lineages. These strains are non-multidrug resistant, have been associated with outbreak in healthcare facilities in other countries and do not usually lead to serious infections. It has also been suggested that t304-ST6 has been imported into Norway through immigration from the Middle East however there is limited epidemiological information available on isolates recovered in Ireland to determine similar links;
- PVL-t002-ST5-MRSA-IV: This strain has been associated with outbreak in two different healthcare facilities. It has recently been described as a Sri Lankan clone and has also been reported in Australia and the UK;
- Other larger outbreaks were caused by t127 (ST1), t1802 (ST5) and t1597 (ST72), all of which have been previously reported in other countries.

In addition to outbreaks of MRSA investigated, a further 100 MSSA isolates were investigated in which 10 clusters were recognised.

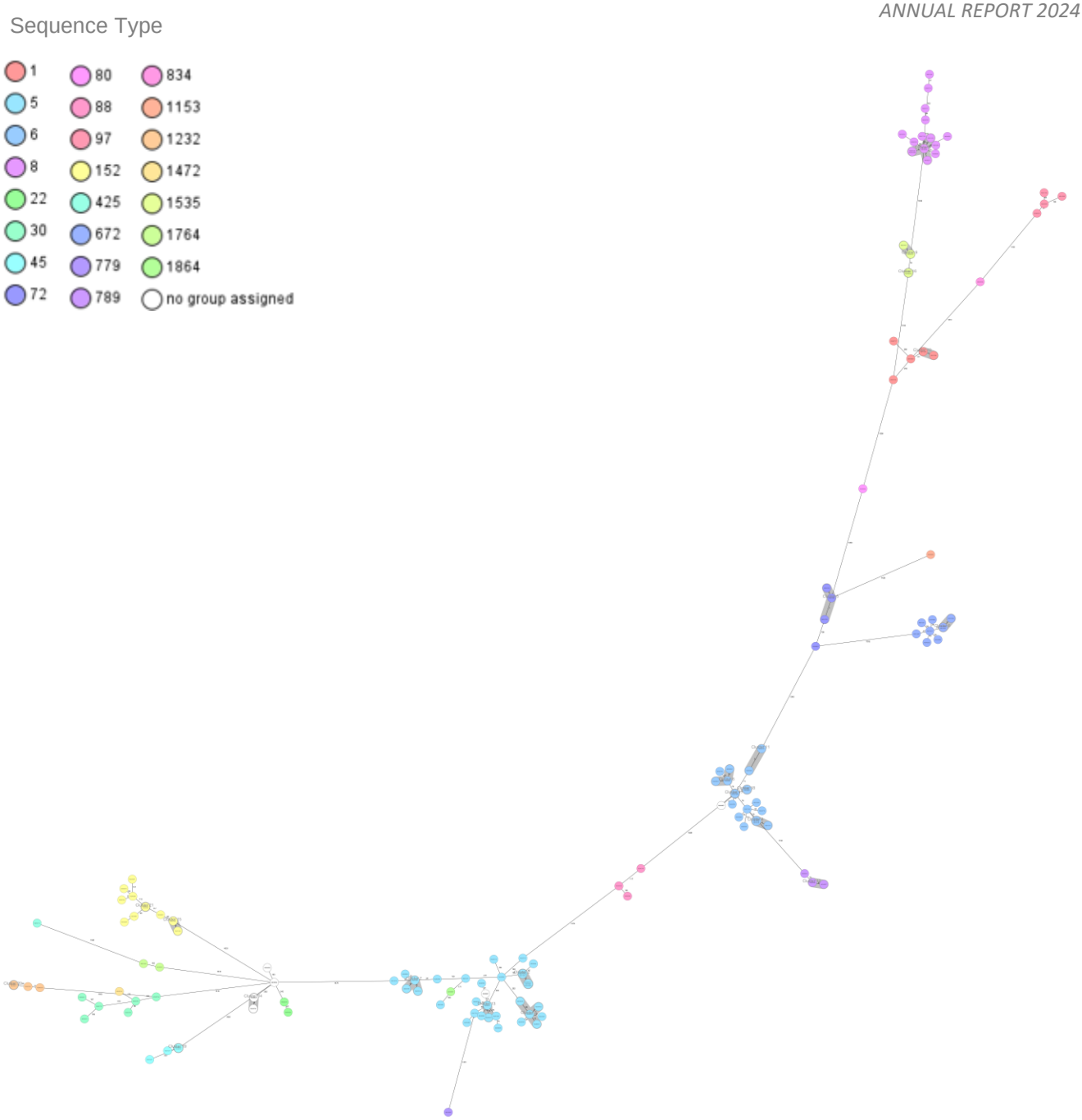


Fig 15: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all MRSA isolates associated with CA-MRSA lineages investigated by whole genome sequencing. In each MST, MRSA isolates assigned to the same sequence type (ST) are indicated by separate colours. Closely related clusters of isolates (≤24 cgMLST allelic differences) are outlined with grey shadowing. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates.

WHOLE GENOME SEQUENCING TO INVESTIGATE VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECIUM* RECOVERED IN IRISH HEALTHCARE SETTINGS

Enterococcus faecium, a resident of the gastrointestinal flora has been a persistent problem in Irish Healthcare settings for many years with vancomycin resistance exhibited by 21% of the isolates recovered from blood stream infections in 2024 (1). For several years the NMRSARL has collaborated with colleagues in the Dublin Dental Hospital to investigate the population structure of VR*Efm* from Irish hospitals using WGS, to explore diversity by investigating the vanA region and to identify potential characteristic features associated with Irish VR*Efm* in relation to the global populations (8).

This study found that all isolates were assigned to the hospital-adapted clade in ST80 but cgMLST assigned the isolates into 51 different clusters which included isolates from different hospitals and from both screening and blood stream infections, suggesting that the population is highly polyclonal. Within clusters, isolates were closely related (8). In investigating the *vanA* operon the study found that the majority of isolates harboured the highly similar *vanA* regions. A comparison of Irish isolates with an international collection showed very little overlap of populations (8).

In recent years the MRSARL has been assisting hospitals investigating outbreaks of VR*Efm* and in 2024 investigated 138 isolates from various different hospitals. The majority of isolates were assigned to ST80 and clonal complex CC17. Among the isolates there were 15 clusters recognized containing varying numbers of isolates. The remaining isolates were unrelated.

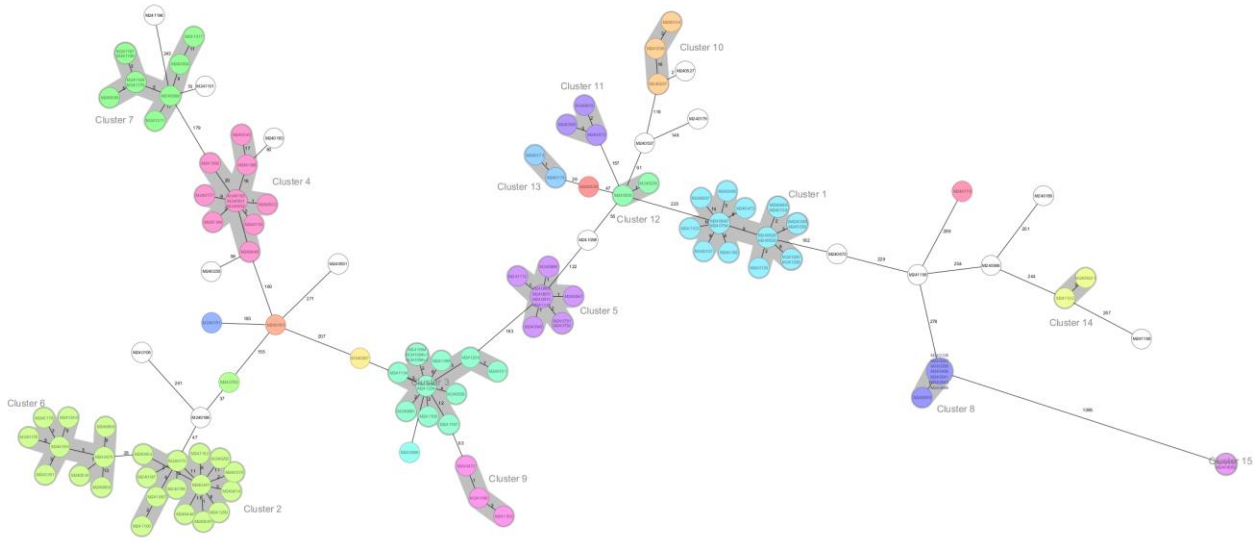


Fig 16: Minimum spanning tree (MSTs) based on core-genome multi-locus sequencing typing (cgMLST) analysis of all VRE isolates investigated by whole genome sequencing. Closely related clusters of isolates (≤24 cgMLST allelic differences) are outlined with grey shadowing. The numbers on each branch indicate the numbers of cgMLST allelic differences detected between neighbouring isolates.

EDUCATION

The NMRSARL plays a prominent role in the education of laboratory staff and clinical staff.

NMRSARL staff gave lectures to undergraduate and post graduate students in the Dept. of Clinical Microbiology, TCD. Scientific staff shared techniques used in the NMRSARL with staff from other hospital laboratories, research facilities, undergraduate students, transition year students and provided expert knowledge to students of other laboratories completing higher degrees.

The laboratory also assisted in several post graduate students undertaking projects including epidemiological typing of MRSA recovered from maternity hospitals, investigation of CoNS and MSSA from diabetic patients and characterisation of CA-MRSA.

CONTINUOUS PROFESSIONAL DEVELOPMENT

The level of expertise and knowledge among staff of NMRSARL is maintained through the participation of staff at both national and international meetings, workshops, and conferences. Throughout the year all staff continued their professional development through attending some of the following meetings;

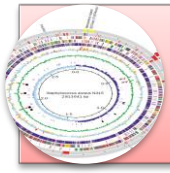
- Journal clubs
- Focus on Infection
- Antimicrobial Resistance
- Microbiology Advisory Body

NMRSARL staff also ensured mandatory training requirements were met in areas such as;

- Risk Management
- Chemical safety awareness
- Manual Handling & Fire safety
- Quality Management
- Hand Hygiene
- Transport of patient specimens

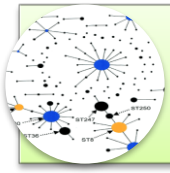
RESEARCH HIGHLIGHTS

NMRSARL continues to participate in several collaborations with both local and international groups to enhance research in the field of *S. aureus* in Ireland.



Whole genome sequencing

- Evaluation of core genome MLST typing methods for the routine use of WGS in outbreak situations



Emerging MRSA strains

- Monitoring of the characteristics of novel and potentially emerging MRSA clones e.g. ST772-MRSA-V, and ST1-MRSA-IV which carry multiple resistance and virulence genes and have been involved in outbreaks in healthcare facilities

CA-MRSA

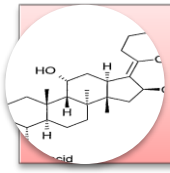
- Characterisation of the genotypes, virulence and antimicrobial resistance genes of *pvl*-positive MRSA in Ireland and MRSA in closed communities

LA-MRSA

- Investigation of MRSA from animal populations for the presence of *mecC* in order to determine if isolates harbouring this gene are a significant problem among LA-MRSA isolates Ireland or if the zoonotic spread of these MRSA strains are contributing to the burden of MRSA among humans

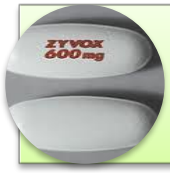
MSSA

- Investigating the genotypes, virulence and antimicrobial resistance potential of MSSA isolates associated with BSI) and MRSA from BSIs in order to investigate why MSSA BSIs are increasing in Ireland while MRSA BSIs are decreasingMupirocin resistance



Fusidic acid resistance

- Investigation of the genetic mechanism of fusidic acid resistance in MRSA in Ireland



Linezolid resistance

- Investigation of linezolid resistance among MRSA, CoNS and VRE and particularly resistance encoded for by the *cfr* and *optrA* genes

PUBLICATIONS

Below are abstracts resulting from these very successful collaborations which have been published or accepted for publication throughout the year.

Brady D, Brennan G, O'Connell B, Buckley R, Brennan M, Lenehan M, Jerry J, Nolke L, Hossein Javadpor S, Hannan M, Lynch B and M Lynch. A 4-year outbreak of MRSA ST72-MRSA-IV *spa* type t1597 in a surgical high dependency unit in Ireland linked to repeated healthcare worker recolonisation. Infect Prev Pract. 2024 Nov 15;7(1):100421.

Abstract

Background: Patients undergoing cardiac surgery are identified as high risk for Staphylococcus aureus infection, including MRSA. An outbreak of MRSA was identified when two patients experienced MRSA infection concurrently in a cardiothoracic high dependency unit with uncommon detection of MRSA previously and an established screening programme.

Methods: An outbreak control team was convened and interventions applied including refresher training in hand and environmental hygiene, review of practice with regard to aseptic access of medical devices and consideration of antibiotic use in the unit. MRSA isolates were referred to the Irish National MRSA Reference Laboratory where spa typing assigned all isolates to t1597 and whole genome sequencing assigned them to multilocus sequence type ST72-MRSA-IV. Recovery of this strain from only this unit in Ireland and infrequent reporting in Europe prompted staff MRSA screening with two staff members found to harbour the outbreak strain. Despite successful decolonisation, recolonisation and further transmission to patients occurred.

Conclusions: In the clinical unit in which this outbreak occurred, the usual control measures to prevent spread of MRSA were in place. Recent Joint Healthcare Infection Society and Infection Prevention Society Guidance does not recommend routine staff screening for MRSA but does support its consideration in an outbreak of an unusual strain. In total, 9 patients and 2 staff were affected by this outbreak. There were 4 infections and 3 deaths. Sustained outbreak closure was necessary to protect certain national clinical programmes and was achievable only when colonised staff were no longer working in the unit.

Traynor R, Brennan GI, Hoban T, Dolan AM, Boyle B, O' Connell B, Shelley O, Teoh TK. Successful control of an outbreak of Panton-Valentine leucocidin positive meticillin resistant *Staphylococcus aureus* in a National Burns Unit through early detection by whole genome sequencing. Infect Prev Pract. 2024 Sep 24;6(4):100400.

Abstract

We report an outbreak of PVL-producing MRSA in the Irish National Burns Unit in 2022 involving seven patients, two staff members and two positive environmental samples. This outbreak was successfully controlled using a range of measures including staff screening, environmental screening and enhanced cleaning. The use of real time whole genome sequencing (WGS) allowed for rapid identification of relatedness and for a rapid outbreak response. We share our successful approach to control this outbreak.

Kavanagh NL, Kinnevey PM, Egan SA, McManus BA, O'Connell B, Brennan GI, Coleman DC. Protracted transmission and persistence of ST80 vancomycin-resistant *Enterococcus faecium* clonal complex types CT2933, CT2932 and CT1916 in a large Irish hospital: a 39-month whole-genome sequencing study. J Hosp Infect. 2024 Sep; 151:11-20. doi: 10.1016/j.jhin.2024.06.002. Epub 2024 Jun 27. PMID: 38944282.

Abstract

Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) are significant nosocomial pathogens. Sequence type (ST) 80 *vanA*-encoding VREfm predominate in Irish hospitals, but their transmission is poorly understood.

Aims: To investigate transmission and persistence of predominant complex type (CT) VREfm in two wards of an Irish hospital (H1) using whole-genome sequencing, and their intra- and inter-hospital dissemination.

Methods: Rectal screening (N = 330, September 2019 to December 2022) and environmental (N = 48, November 2022 to December 2022) *E. faecium* were investigated. Isolate relatedness was assessed by core-genome multi-locus sequence typing (cgMLST) and core-genome single nucleotide polymorphism (cgSNP) analysis. Likely transmission chains were identified using SeqTrack (https://graphsnp.fordelab.com/graphsnp) using cgSNP data and recovery location. Well-characterized E. faecium (N = 908) from seven Irish hospitals including H1 (June 2017 to July 2022) were also investigated.

Findings: Conventional MLST assigned isolates to nine STs (ST80, 82%). cgMLST identified three predominant ST80 CTs (CT2933, CT2932 and CT1916) (55% of isolates) of related isolates (≤20 allelic differences). cgSNP analysis differentiated these CTs into multiple distinct closely related genomic clusters (≤10 cgSNPs). Parisimonious network construction identified 55 likely inter- and intra-ward transmissions with epidemiological support between patients ≤30 days involving 73 isolates (≤10 cgSNPs) from seven genomic clusters. Numerous other likely transmissions over longer time periods without evident epidemiological links were identified, suggesting persistence and unidentified reservoirs contribute to dissemination. The three CTs predominated among *E. faecium* (N = 1286) in seven hospitals, highlighting inter-hospital spread without known epidemiological links.

Conclusion: This study revealed the long-term intra- and inter-hospital dominance of three major CT ST80 VREfm lineages, widespread transmission and persistence, implicating unidentified reservoirs.

RESOURCES

Staff

During 2024 the staff working in the NMRSARL were:

- Gráinne Brennan
- Tanya Fleming
- Paul Grier
- Ludmila Fadejeva
- John Eakins

The role of Director was discharged in an honorary capacity by Dr. Brian O'Connell, Consultant Microbiologist, SJH.

Tanya Fleming passed away during the year and we take this opportunity to acknowledge her contribution to the laboratory and the wider medical science field during her career.

Administration

The laboratory is in St. James's Hospital and is administered within the Laboratory Medicine (LabMed) Directorate.

Facilities

NMRSARL consists of three main laboratory areas, a Phenotyping Laboratory, a Genotyping Laboratory and a PCR Laboratory. The provision of a suitable computer system is a major requirement, both for monitoring isolates received and for detailed analytical work.

Along with the Central Pathology Laboratory in SJH, NMRSARL has been involved in procuring a new computer system for several years and as part of this procurement, the special requirements of NMRSARL have been noted. However, all systems investigated to date would require extensive modification to accommodate NMRSARL's needs.

Finance

The budget allocated to the NMRSARL for the year to cover both pay and non-pay elements amounted to €325,000.

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